JOURNAL OF CLINICAL MICROBIOLOGY, July 1978, p. 36-41
0095-1137/78/0008-0036$02.00/0

Vol. 8, No. 1
Printed in U.S.A.

Improved Media for Growth and Aerotolerance of *Campylobacter fetus*

HUGH A. GEORGE,[1]* PAUL S. HOFFMAN,[1] ROBERT M. SMIBERT,[2] AND NOEL R. KRIEG[1]

Department of Biology (Microbiology Section)[1] and the Anaerobe Laboratory,[2] Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

Received for publication 11 May 1978

The microaerophilic nature of *Campylobacter fetus* has complicated its recovery from human and animal sources. In this study, modifications of brucella agar and broth were tested for enhancement of growth and aerotolerance of 64 strains of *C. fetus*, representing each subspecies. Brucella agar supplemented with 0.025% each $FeSO_4 \cdot 7H_2O$, sodium metabisulfite, and sodium pyruvate, supported growth of 98, 77, and 63% of the strains at 6% O_2, 17% O_2, and 21% O_2, respectively. Unsupplemented brucella agar supported growth of 94, 48, and 20% of the strains. Brucella broth supplemented with 0.2% $FeSO_4 \cdot 7H_2O$, 0.025% sodium metabisulfite, and 0.05% sodium pyruvate supported growth of 98% of the strains at 21% O_2, compared to 75% with unsupplemented brucella broth. With both the supplemented agar and broth, growth responses occurred 1 to 2 days earlier than usual. Growth and aerotolerance of three strains of *Campylobacter sputorum* subsp. *bubulus* were not enhanced by the supplements.

Campylobacter fetus requires oxygen for growth, yet is inhibited by oxygen at normal atmospheric partial pressure (3, 6). Growth occurs at oxygen concentrations below 21%, and even candle jar conditions (17% O_2) may provide too much oxygen for some strains. The optimum concentration of oxygen has been reported to be 6% (3). The microaerophilic nature of *C. fetus*, as well as a requirement for carbon dioxide (3), have complicated recovery of the organism from human and animal sources.

Catalase has been reported to stimulate the growth of *C. fetus* (4). Border et al. (1) found that addition of hematin to brucella agar allowed growth of some strains in air containing 5% CO_2. Bowdre et al. (2) found that addition of low levels of norepinephrine, or high levels of iron salts, to brucella agar permitted *C. fetus* subsp. *jejuni* to grow at 21% O_2 plus 2.5% CO_2.

In the present study the ability of a combination of ferrous sulfate, sodium bisulfite, and sodium pyruvate to enhance the aerotolerance of *C. fetus* was evaluated for 64 strains, representing each subspecies.

MATERIALS AND METHODS

Source of strains and maintenance of stock cultures. Sixty-four strains of *C. fetus* from human and animal sources were used. The strain designations and sources are indicated in Tables 1 to 3. Also, three strains of *Campylobacter sputorum* subsp. *bubulus* (no. 861, 867, and 53103) from the Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, were used. Stock cultures were maintained in brucella broth (Pfizer Diagnostics) containing 0.15% agar and 0.02% $FeSO_4 \cdot 7H_2O$.

Viable counts. Cultures were grown at 37°C with agitation in brucella broth in an atmosphere containing 6% O_2, 2.5% CO_2, and 92.5% N_2. Log-phase cultures were diluted to a turbidity of 55 Klett units (red filter, 16-mm cuvettes) in brucella broth; this suspension was further diluted by a factor of 10^6. One-tenth milliliter of the final dilution was spread on the surface of agar plates which had previously been dried overnight at room temperature. The media consisted of plain brucella agar and brucella agar supplemented with 0.025% each $FeSO_4 \cdot 7H_2O$, sodium metabisulfite, and sodium pyruvate (FBP agar). The pH of the media was 7.4. Plates were incubated in atmospheres of 6% O_2, 17% O_2, and 21% O_2. Carbon dioxide (2.5%) was provided at each oxygen level. Final partial pressures of gas mixtures were adjusted manometrically to standard sea level values. Triplicate plates for all experiments were incubated for 4 days at 37°C, and the mean colony counts were recorded. The results were expressed as a growth index, defined as the colony counts obtained under the test conditions divided by the counts obtained on unsupplemented brucella agar incubated at 6% O_2 and 2.5% CO_2.

Total counts. For a representative strain of each of the three subspecies of *C. fetus*, colony counts of log-phase cells on brucella agar and FBP agar plates were compared to direct microscopic counts (Petroff-Hausser chamber). The percent recovery of colonies compared to the total numbers of cells was calculated.

Growth in broth. Log-phase cells were grown as indicated above. Approximately 10% cells were inoculated into 50 ml of plain brucella broth and brucella broth supplemented with 0.2% $FeSO_4 \cdot 7H_2O$, 0.025% sodium metabisulfite, and 0.05% sodium pyruvate (FBP broth). The media were contained in square

milk dilution bottles. Cultures were incubated statically at 37°C under 21% O_2 and 2.5% CO_2. Turbidities (in Klett units) were measured after 72 h. The results were expressed as a growth index, defined as the turbidity obtained with FBP broth divided by the turbidity obtained with plain brucella broth.

RESULTS

Viable counts. Results for strains of *C. fetus* subsp. *intestinalis* are presented in Table 1. The most oxygen-sensitive as well as the most oxygen-tolerant strains occurred within this subspecies. At 6% O_2, 34 of 38 strains (89%) grew on brucella agar, whereas 37 strains (97%) grew on FBP agar. Strain 436 was extremely sensitive to oxygen and failed to grow on either medium at 6% O_2; growth occurred only on FBP agar incubated in an atmosphere containing 1% O_2 and 2.5% CO_2. At 17% O_2, 17 of 38 strains (45%) grew on brucella agar, whereas 25 strains (66%) grew

TABLE 1. *Growth responses of C. fetus subsp. intestinalis on brucella agar and FBP agar at various oxygen tensions*

Strain	Source[a]	Growth index[b] with incubation at:				
		6% O_2, 2.5% CO_2 (FBP agar)	17% O_2, 2.5% CO_2		21% O_2, 2.5% CO_2	
			Brucella agar	FBP agar	Brucella agar	FBP agar
2221	Ov	1.0	0.6	1.0	0.0	0.0
13161	Ov	1.0	0.6	1.2	0.0	*0.8*[c]
Grant	Ov	1.0	0.0	0.0	0.0	0.0
Langford	Ov	1.0	0.0	*0.7*	0.0	*0.6*
6339	Ov	NC (49)[d]	NC (0)	NC (0)	NC (0)	NC (0)
4440	Ov	1.1	0.6	0.9	0.0	*0.6*
Keith 76-1435	Bv	1.1	0.0	*0.9*	0.0	*0.8*
DT-74	Bv	0.8	0.0	0.0	0.0	0.0
702	Bv	1.2	0.0	0.0	0.0	0.0
436	Bv	NC (0)	NC (0)	NC (0)	NC (0)	NC (0)
HCB	Bv	1.2	0.8	1.6	0.8	0.9
EMB 1545	Bv	1.1	0.0	*1.0*	0.0	0.0
273	Bv	1.0	0.0	*0.9*	0.0	*0.8*
Nelson 1	Hu	4.5	0.0	0.0	0.0	0.0
10951	Hu	1.1	1.0	1.1	0.5	0.4
319-A-76	Hu	1.1	1.2	1.2	0.0	0.0
10148	Hu	1.0	0.0	0.0	0.0	0.0
Pacemaker	Hu	1.0	0.8	1.0	0.0	*0.8*
Pedro	Hu	1.2	1.2	1.2	1.2	1.2
11669	Hu	1.0	1.0	1.0	1.0	1.1
1510 MB	Hu	1.1	0.0	*1.2*	0.0	*0.9*
11164	Hu	1.0	0.7	0.9	0.0	0.0
PB 1/76	Hu	6.6	0.0	0.0	0.0	0.0
8391-B-76	Hu	1.0	1.1	1.1	1.1	1.0
11529	Hu	1.0	0.4	1.1	0.0	*1.1*
11639-B-76	Hu	1.5	0.5	1.6	0.0	*1.5*
PB 1/35	Hu	0.9	0.0	0.0	0.0	0.0
B6286	Hu	0.9	0.3	1.1	0.0	*0.8*
HFl	Hu	1.0	0.0	0.0	0.0	0.0
PB1/1	Hu	NC (39)	NC (0)	NC (0)	NC (0)	NC (0)
1925	Hu	0.9	0.0	0.0	0.0	0.0
Barr	Hu	1.1	0.0	*0.9*	0.0	0.0
10583	Hu	2.2	0.0	*2.3*	0.0	*1.5*
18151-B-76	Un	1.1	1.2	1.3	0.0	0.0
10451	Un	0.9	1.0	1.1	0.0	0.0
10296	Un	3.2	0.0	*3.4*	0.0	*3.4*
11324-A-76	Un	1.1	1.1	1.1	0.6	1.1
W5-184	Un	NC (42)	NC (0)	NC (0)	NC (0)	NC (0)

[a] Ov, Ovine; Bv, bovine; Hu, human; Un, unknown.

[b] Growth index = the colony counts obtained under the test conditions divided by the colony counts obtained at 6% O_2, 2.5% CO_2 on unsupplemented brucella agar. Results were calculated from the mean values of triplicate plates. Growth indexes less than 0.2 (2 standard deviations) were considered to be 0.0.

[c] Values in italics indicate cases where growth occurred on FBP agar but not on unsupplemented brucella agar.

[d] NC, Not calculated. No growth occurred on brucella agar at 6% O_2, 2.5% CO_2. The numbers in parentheses = number of colonies at a dilution of 10^{-7} on FBP agar.

on FBP agar. Of the animal strains (bovine or ovine) tested, 4 of 13 (31%) grew at 17% O_2 on brucella agar, and 8 strains (62%) grew on FBP agar. Of the human strains tested, 4 of 20 (20%) grew on brucella agar, and 13 strains (65%) grew on FBP agar. At 21% O_2, only 6 of 38 strains (16%) of *C. fetus* subsp. *intestinalis* grew on brucella agar, whereas 18 strains (47%) grew on FBP agar. Of the animal strains, only 1 of 13 strains (8%) grew on brucella agar, whereas 6 strains (38%) grew on FBP agar. Of the human strains, 4 of 20 (20%) grew on brucella agar, whereas 10 strains (50%) grew on FBP agar. The most aerotolerant strains found, i.e., those giving growth indexes of $\geqq 1.0$ on plain brucella agar at 21% O_2, were all obtained from human sources.

The results for strains of *C. fetus* subsp. *jejuni* are indicated in Table 2. All 18 strains grew on both brucella agar and FBP agar at 6% O_2. At 17% O_2, however, only 13 strains (72%) grew on brucella agar, whereas all 18 strains (100%) grew on FBP agar. At 21% O_2, only 7 strains (39%) grew on brucella agar, whereas all 18 strains (100%) grew on FBP agar.

The results for *C. fetus* subsp. *fetus* are indicated in Table 3. All 8 strains grew on brucella agar and FBP agar at 6% O_2. Only 1 of 8 strains (13%) was capable of growth on plain brucella agar at 17% O_2, and no strains were capable of growth at 21% O_2. As with the other subspecies, FBP agar enhanced aerotolerance, with 6 of 8 strains (75%) being capable of growth on this medium at 17% O_2 and 4 strains (50%) being capable of growth at 21% O_2.

Considering the three subspecies together, FBP agar supported growth of 63 of 64 strains (98%) at 6% O_2, whereas 60 strains (94%) grew on plain brucella agar. The single strain (no. 436) that did not grow on either medium at 6% O_2 was able to grow at 1 to 3% O_2, but only on FBP agar. At 17% O_2, FBP agar supported the growth of 49 of 64 strains (77%), whereas only 31 strains (48%) grew on plain brucella agar. At 21% O_2, FBP agar supported the growth of 40 of 64 strains (63%), whereas plain brucella agar supported growth of only 13 strains (20%). None of the strains of *C. fetus* could grow under strictly anaerobic conditions.

Viable counts versus total counts. For three strains of *C. fetus* grown on brucella agar and FBP agar at 6% O_2, the colony count per milliliter was compared to the total count per milliliter as estimated by direct microscopic count. For *C. fetus* subsp. *intestinalis* strain 1510 MB, the colony count per milliliter was 45% of the direct microscopic count per milliliter when plain brucella agar was used versus 86% when FBP agar was used. Similarly, for *C. fetus* subsp.

TABLE 2. *Growth responses of C. fetus subsp. jejuni on brucella agar and FBP agar at various oxygen tensions*

Strain	Source[a]	Growth index[b] with incubation at:				
		6% O_2, 2.5% CO_2 (FBP agar)	17% O_2, 2.5% CO_2		21% O_2, 2.5% CO_2	
			Brucella agar	FBP agar	Brucella agar	FBP agar
8916	Ov	1.1	0.8	1.1	0.0	*0.8*[c]
13136	Ov	1.1	0.7	0.8	0.0	*0.3*
4849	Ov	1.1	0.0	*0.9*	0.0	*0.3*
Wang III	Fe	1.0	0.0	*0.9*	0.0	*0.8*
C14	Ch	1.3	0.6	1.6	0.4	1.1
29A	Ch	1.0	1.0	0.9	0.5	0.9
Otis P.	Hu	1.0	1.4	1.4	0.0	*1.1*
6963	Hu	1.0	0.9	1.1	0.0	*0.7*
H641	Hu	1.0	0.9	1.0	0.7	0.8
B7619	Hu	1.2	0.0	*1.3*	0.0	*1.1*
H840	Hu	1.4	0.0	*1.2*	0.0	*1.0*
Smith	Hu	1.0	0.9	1.1	1.0	1.1
H550	Hu	0.9	1.3	1.3	1.0	0.9
11642-B-76	Hu	1.1	0.9	1.0	0.8	0.9
H325	Hu	1.0	0.8	0.7	0.4	0.8
Holy Cross	Hu	1.1	0.6	0.9	0.0	*0.7*
11641-B-76	Hu	1.0	0.8	0.9	0.0	*0.7*
8945-A-76	Un	0.8	0.0	*0.7*	0.0	*0.3*

[a] Ov, Ovine; Fe, feline; Ch, chicken; Hu, human; Un, unknown.

[b] Growth index = the colony counts obtained under the test conditions divided by the colony counts obtained at 6% O_2, 2.5% CO_2 on unsupplemented brucella agar. Results were calculated from the mean value of triplicate plates. Growth indexes less than 0.2 (2 standard deviations) were considered to be 0.0.

[c] Values in italics indicate cases where growth occurred on FBP agar but not on unsupplemented brucella agar.

TABLE 3. *Growth responses of C. fetus subsp. fetus on brucella agar and FBP agar at various oxygen tensions*

Strain	Source[a]	Growth index[b] with incubation at:				
		6% O_2, 2.5% CO_2 (FBP agar)	17% O_2, 2.5% CO_2		21% O_2, 2.5% CO_2	
			Brucella agar	FBP agar	Brucella agar	FBP agar
21085	Bv	1.1	0.0	0.0	0.0	0.0
14093	Bv	0.9	0.3	0.9	0.0	*0.9*[c]
7721	Bv	1.4	0.0	*0.7*	0.0	*0.3*
75-183	Bv	3.2	0.0	*2.1*	0.0	*1.1*
13841	Bv	1.4	0.0	*0.9*	0.0	*0.4*
14664	Bv	2.0	0.0	*1.1*	0.0	0.0
14701	Bv	1.5	0.0	0.0	0.0	0.0
998	Bv	1.4	0.0	*0.3*	0.0	0.0

[a] Bv, Bovine.

[b] Growth index = the colony counts obtained under the test conditions divided by the colony counts obtained at 6% O_2, 2.5% CO_2 on unsupplemented brucella agar. Results were calculated from the mean value of triplicate plates. Growth indexes less than 0.2 (2 standard deviations) were considered to be 0.0.

[c] Values in italics indicate cases where growth occurred on FBP but not on unsupplemented brucella agar.

jejuni strain H840 and *C. fetus* subsp. *fetus* strain 998, values of 85 versus 98% and 66 versus 84%, respectively, were obtained.

Growth on streaked plates. On plates streaked by the "T" method (2) and incubated at 6% O_2, *C. fetus* strains generally formed colonies on the first (most heavily inoculated) and second sections of the plate by day 2 when FBP agar was used but only on the first section of the plate when brucella agar was used. For any given strain, colony diameters were smaller on brucella agar than on FBP agar. On FBP agar the various strains had colony diameters ranging from 0.4 to 1.0 mm, whereas colonies on brucella agar ranged from 0.3 to 0.7 mm. By day 3 colonies were evident on all three sections of FBP agar plates but only on the first two sections of brucella agar plates. Colony diameters on FBP agar now ranged from 0.9 to 1.4 mm, whereas those on brucella agar ranged from 0.5 to 1.1 mm. By day 4 colonies were present on all three sections of the plates of both media, and the colony diameters for any given strain were now similar on both media. The colony diameters ranged from 1.0 to 1.8 mm. In comparing the three subspecies of *C. fetus,* the colonies of *C. fetus* subsp. *fetus* were consistently smaller than the colonies of the other two subspecies.

Regardless of size, colonies formed on FBP agar by the various strains of *C. fetus* were round, entire, convex, translucent, and smooth and glistening and ranged in color from white to light tan.

During the past year, 35 fresh clinical isolates of *C. fetus* were received by our laboratory for confirmation of identification. Upon receipt, each strain was purified by streaking on FBP agar with subsequent incubation at 6% O_2. In every case, FBP agar supported an excellent growth response. In certain cases where the culture received by us contained very few viable cells, growth occurred on FBP agar but not on plain brucella agar.

Growth in broth. FBP broth supported the growth of 63 of 64 strains of *C. fetus* at 21% O_2 with static incubation (Tables 4 and 5). Only strain 436 of *C. fetus* subsp. *intestinalis,* previously found to be highly oxygen sensitive on agar plates, failed to grow in FBP broth. In contrast to FBP broth, brucella broth supported the growth of only 48 of the 64 strains. Where both brucella broth and FBP broth supported growth, maximum turbidity of the cultures developed 1 to 2 days earlier in the FBP broth.

C. sputorum. When tested at 6% O_2, three strains of *C. sputorum* subsp. *bubulus* grew on both brucella agar and FBP agar, and colony counts were similar on both media. The strains failed to grow on either medium when tested at 17 and 21% O_2. Thus, the oxygen tolerance of *C. sputorum* appears not to be increased by the supplements added to the brucella agar.

DISCUSSION

Although FBP agar enhanced aerotolerance of all strains tested in this study, and although nearly all strains could grow in FBP broth at 21% O_2, the variety of levels of aerotolerance among the various strains makes it unlikely that all strains of *C. fetus* can be isolated or cultivated on solid media at 17 or 21% O_2. However, FBP agar does offer advantages over plain brucella agar. In laboratories where a candle jar might be the only available means for reducing oxygen tensions and providing carbon dioxide for isolation of *C. fetus,* a greater proportion of strains would be recovered on FBP agar compared to plain brucella agar (77% compared to 48% of the

TABLE 4. *Growth of C. fetus subsp. intestinalis in FBP broth at 21% O_2, 2.5% CO_2*

Strain	Growth index[a]	Strain	Growth index
2221	1.9	11669	1.7
11361	1.3	1510 MB	1.7
Grant	NC (10)[b]	11164	1.4
Langford	NC (13)	PB 1/76	NC (13)
6339	NC (24)	8391-B-76	1.2
4440	1.8	11529	1.4
Keith 76-1435	3.0	11639-B-76	1.3
DT-74	NC (15)	PB 1.35	1.4
702	1.2	B6286	1.2
436	NC (0)	HF1	7.0
HCB	NC (5)	PB 1/1	NC (21)
EMB 1545	NC (13)	1925	NC (17)
273	1.2	Barr	1.6
Nelson I	NC (24)	10583	2.5
10951	1.1	18151-B-76	1.3
319-A-76	1.6	10451	1.7
10148	1.8	10296	1.4
Pacemaker	NC (22)	11324-A-76	1.7
Pedro	2.3	W5-184	3.0

[a] Growth index = the turbidity reached in FBP broth at 72 h divided by the turbidity reached in brucella broth.

[b] NC, Not calculated. No growth occurred in brucella broth. The value given in parentheses = the turbidity in Klett units reached in FBP broth.

TABLE 5. *Growth of C. fetus subsp. jejuni and C. fetus subsp. fetus in FBP broth at 21% O_2, 2.5% CO_2*

Organism	Strain	Growth index[a]
C. fetus subsp. *jejuni*	8916	1.0
	13136	1.2
	4849	NC (11)[b]
	Wang III	1.4
	C14	1.8
	29A	1.3
	Otis P.	3.0
	6963	1.5
	H641	1.4
	B7619	1.5
	H840	1.2
	Smith	1.4
	H550	1.4
	11642-B-76	2.3
	H325	1.4
	Holy Cross	3.2
	11641-B-76	2.3
	8945-A-76	1.2
C. fetus subsp. *fetus*	21085	NC (14)
	14093	2.2
	7721	1.4
	75-183	2.1
	13841	NC (13)
	14664	2.3
	14701	NC (25)
	998	NC (18)

[a] Growth index = the turbidity reached in FBP broth at 72 h divided by the turbidity reached in brucella broth.

[b] NC, Not calculated. No growth occurred in brucella broth. The value given in parentheses = the turbidity in Klett units reached in FBP broth.

strains in the present study). Even at 6% O_2, where most strains can be recovered on either FBP agar or brucella agar, FBP agar offers advantages. Three strains in the present study which were not capable of growth on plain brucella agar at 6% O_2 could grow on FBP agar. Therefore, extremely oxygen-sensitive strains may be recoverable on FBP agar under conditions where they could not be recovered on brucella agar. Another advantage of FBP agar (and also FBP broth) is the greater rapidity with which growth appears. In general, growth occurs 1 to 2 days earlier on the FBP media.

The results of this study indicate that for the most reliable isolation and cultivation of *C. fetus* on solid media, FBP agar should be used. Incubation of streaked plates should be in a jar containing a mixture of 3 to 6% O_2, 2.5% CO_2, and 91 to 95% N_2. Visible colony formation would generally occur within 2 to 3 days, even with highly oxygen-sensitive strains.

The failure of *C. sputorum* to exhibit an increased oxygen tolerance with FBP agar is in agreement with the report by Neikus et al. (5). *C. sputorum* is probably not an aerobe or microaerophile but is instead an aerotolerant anaerobe. This is suggested by the results of Van Palenstein-Helderman and Rosman (7) and Neikus et al. (5) and also by unpublished work in our laboratory. *C. sputorum* is similar in its energy metabolism to *Vibrio succinogenes* in that it grows anaerobically with hydrogen or formate as an electron donor and fumarate or nitrate as an electron acceptor.

The function of $FeSO_4$, bisulfite, and pyruvate in enhancing oxygen tolerance in *C. fetus* is under investigation.

ACKNOWLEDGMENT

This investigation was supported by Public Health Service grant G.M.S. 14604 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Border, M. M., B. D. Firehammer, and L. L. Myers.** 1974. Tube culture method for viable counts of *Campylobacter fetus* (*Vibrio fetus*). Appl. Microbiol. **28**:730–732.
2. **Bowdre, J. H., N. R. Krieg, P. S. Hoffman, and R. M. Smibert.** 1976. Stimulatory effect of dihydroxyphenyl compounds on the aerotolerance of *Spirillum volutans* and *Campylobacter fetus* subsp. *jejuni*. Appl. Environ. Microbiol. **31**:127–133.
3. **Kiggins, E. M., and W. N. Plastridge.** 1956. Effect of gaseous environment on growth and catalase content of *Vibrio fetus* cultures of bovine origin. J. Bacteriol. **72**:397–400.

4. **Kingscote, B.** 1961. Use of catalase in the culture of *Vibrio fetus*. Can. J. Microbiol. **7**:951-952.
5. **Neikus, H. G. D., W. DeVries, and A. H. Stouthamer.** 1977. The effect of different dissolved oxygen tensions on growth and enzyme activities of *Campylobacter sputorum* subspecies *bubulus*. J. Gen. Microbiol. **103**:215-222.
6. **Smibert, R. M.** 1974. Genus II. *Campylobacter* Sebald and Véron 1963, 907, p. 207-212. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
7. **Van Palenstein-Helderman, W. H., and I. Rosman.** 1976. Hydrogen-dependent organisms from the human gingival crevice resembling *Vibrio succinogenes*. Antonie van Leeuwenhoek J. Microbiol. Serol. **42**:107-118.